MULTIPLE INFECTIONS OF PARASITES

BY

P. C. C. GARNHAM

As physicians, we tend to think anthropocentrically in regard to parasitic infections of man, and if we live in temperate countries, where parasitic conditions are much less prevalent than in the tropics, we usually assume that a single species of parasite is responsible for the disease — malaria, hookworm, trichinellosis, relapsing fever, etc.

But when we move further south and come into contact with more primitive conditions, the patients are often found to be parasitised with multiple infections — two or three species of *Plasmodium*, *Entamoeba histolytica*, *Taenia saginata*, *Schistosoma mansoni* and various commensals. The neat picture presented by one parasite in Europe or North America is now much blurred, one species influencing another and altering the pathology in the host.

As zoologists, we shy away from the human angle, and realize that in animals, multiple infections are the rule and not the exception even in temperate climates, but naturally they are more pronounced in the warmer parts of the globe. You have only to examine a few sparrows, for instance, to find perhaps three haematozoa in the blood, several coccidia and helminths in the intestinal tract, and others in the tissues. If we cast the net still wider, and include bacteria, viruses, spirochaetes, etc. as parasites, as you do in France, the spectrum becomes even more complex, and the disentanglement of the separate threads may be a great problem.

This is a subject, about which we all are aware, but few like to tackle. When my late, lamented colleague, Ronald Heisch returned from the tropics to join me in London I suggested to him that here was an interesting research project, but the scientific mind is much more drawn to the solution of a single life cycle, the characters of a new species, or even such applied aspects as the epizootiology of the infection, and I failed to persuade him.

Yet the subject is clearly open to scientific investigation, starting with a clean animal, and adding a single parasite and then giving secondary infections ; the effects are observed and the various changes noted. One looks for two principal effects — behavioural and morphological — but there is a third character that is probably of immense practical importance — the immunological aspect. The immunity mechanism may become so exhausted by the response to one parasite that the host is unable to cope with the ravages of another. Young African children become heavily infected with malaria parasites, their weapons of defence are put out of action and the child dies from pneumococcal infections — at least this is the commonly held assumption.

In many of the experiments that we carry out, the existence of mixed infections may not be recognised, because specific parasite-free animals, are rarely used or still less, totally parasite-free ones. The excuse for the failure to employ such precautions is either financial (such animals are very expensive) or that the conditions of the experiment are far from natural (where multiple infections are the rule).

In this paper I want to discuss a few specific parasitic associations which I have come across, in order to indicate some of the more puzzling situations.

1. *P. falciparum* and *P. vivax* (plus *Treponema pallidum*)
2. *P. inui* and *Babesia microti*.
3. Rodent malaria and *Eperythrozoon*.
4. Mixed infections of malaria parasites in man.
5. Mixed infections of malaria parasites in birds.
6. Multiple infections of parasites in geckos.
7. The malign intrusion of viruses.

1. *The influence of* P. falciparum *on the course of* P. vivax *and other parasites.* These malaria parasites were used for many years in the treatment of general paralysis of the insane. This disease is caused by a third parasite — *Treponema pallidum* ; the effect of its presence has been ignored in research on malaria parasites subsequently introduced. (Incidentally, the *Plasmodium* has a damaging effect on the spirochaete, and has held to be responsible for the rarity of tertiary syphilis in Africa, where malaria is universal).

But of extraordinary interest is the effect of *P. falciparum* on *P. vivax* when the sporozoites of both species are introduced simultaneously, as was done by Shute (1946) in the Malaria Therapy Unit of Horton Hospital. Fever breaks out 10 days later, but only *P. falciparum* appears in the blood ; several months later however *P. vivax* suddenly invades the blood and bening tertian fever follows. If however the *P. vivax* sporozoites are given 3 or 4 days before the *P. falciparum*, parasites of both species appear together in the blood stream. The explanation of the inhibition still eludes us.

P. falciparum is apparently capable of suppressing *P. malariae* in nature as was shown by a W.H.O. team (Molineaux *et al.* 1980) in Northern Nigeria, where both species are prevalent but there is a 30 week time lag between the onset of parasitaemia due to the quartan parasite and the initial overwhelming bout of *P. falciparum*. These workers ascribe it, partially at least, to the operation of heterologous immunity, but the explanation is invalidated, because, in experimental studies, the inoculation of the blood forms (of the suppressed species) is immediately followed by parasitaemia.

2. *Mixed infections of* Babesia microti *and* Plasmodium inui *and other piroplasm : malaria combinations.* On a recent visit to the Chamblee Laboratory (Communicable Diseases Center, Atlanta), Dr. William Chin, kindly showed me (and has allowed me to mention today) certain experiments which apparently demonstrate a similar form of behavioural change as the above, but in different orders of parasites. A rhesus monkey was bitten by ticks infected with *B. microti*, but no parasites appeared in the blood and 18 months later, the same monkey was infected with *P. inui* by blood inoculation. The malaria parasite multiplied normally, and the blood was inoculated into three Saimiri monkeys which developed heavy *P. inui* infections. At last, and two years after the original infective tick bite (in another monkey), the babesial infection became patent, fulminated and killed the 3 Saimiris, at the same time exterminating the malaria.

This rather complex series of events, in which immunity may have played a part, is best viewed against the simpler experimental work of Cox (1970, 1972, and 1975) and others using *Plasmodium berghei* (and other species), *Babesia microti* (and *B. rodhaini*) and *Anthemosoma garnhami*. The piroplasms and the malaria parasites were unable to co-exist because there is a high degree of reciprocal immunity ; the dactylosome however confers less protection especially on *P. berghei* and *P. yoelii*. Cox points out that the interaction between these intraerythrocytic parasites may influence their incidence in the wild.

3. *Rodent malaria and* Eperythrozoon. Most workers on rodent malaria have been incommoded at times, because of the presence of this rickettsia like organism in their experimental animals. The condition becomes exacerbated after splenectomy and various investigators (e.g. Peters 1965) have drawn attention to the fallacies which may arise in chemotherapeutic studies by failure to recognise the inhibitory influence of *E. coccoides* on the growth of the parasite. Cox (1970) discusses various factors which might influence this process by *Eperythrozoon* or *Haemobartonella muris* ; the obvious mode of action would appear to be that the coating of the erythrocyte by *Eperythrozoon* suffices to seal its surface from invasion by the malaria parasite.

4. *Mixed infections of malaria parasites in man.* Infections with two or three of the human

species of *Plasmodium* are quite common in holoendemic areas of malaria in tropical Africa and even rarely all four species have been identified with some cerainty in a single thin blood film — the fourth parasite being *P. ovale*. This species was at one time thought to be a cross between *P. vivax* and *P. malariae*, as it shares some of the characters of both. However, *P. ovale* possesses three features which serve to distinguish it from *P. vivax* — distinctive pigment pattern in the oocyst, characteristic exoerythrocytic cycle and infectivity to the negro (to whom *P. vivax* is insusceptible). As far as I know, no attempts have been made to hybridise *P. vivax* and *P. malariae* in mosquitoes. I have never observed in Africa any morphological changes induced by the presence of multiple parasites and the curious behavioural effects referred to above are not easily detected in nature.

Similarly, the presence of other parasites such as relapsing fever spirochaetes, trypanosomes, microfilaria in the blood of patients with malaria has been unaccompanied by any obvious changes in the parasites. The only exception may be the apparent absence of human babesiosis in the malarious tropics, where the universal malaria may protect the inhabitants against the tick-transmitted infection. Incidentally, the differential diagnosis between *Babesia* spp. and *P. falciparum* may be quite difficult.

5. *Multiple infections in birds.* I have already drawn attention to the frequency with which multiple infections occur in wild birds. Seasonal transmission tends to keep the parasites apart, even for short intervals, while genera which are transmitted by ectoparasitic vectors, such as *Haemoproteus* and hippoboscid flies are likely to be non-seasonal, at least in the tropics.

Numerous surveys of Haematozoa in wild birds have been made and the International Reference Centre at the University of Newfoundland has prepared detailed check-lists of *Haemoproteus*, *Plasmodium*, microfilaria, *Trypanosoma*, haemogregarines (including *Atoxoplasma*) and *Leucocytozoon*. These genera are listed in order of relative prevalence, with a total of 3,743 infections in 35,555 Neotropical birds (White *et al.* 1978). Multiple infections were frequent. There was no relationship between the prevalence of parasites and avian phylogeny, nor correlation between the phylogeny of the genera of parasites in the various orders of birds.

Of greater interest to this Symposium are the results of investigations of greater depth, and I should like to refer particularly to the work of Gabaldon and Ulloa (1980) who for several years have been studying avian malaria in limited areas in the llanos of Venezuela. They found a concentration of nestling birds of the order Ciconiiformes with a 100 % infection rate of various species of *Plasmodium*. A single mosquito vector — *Aedomyia squamipennis* was concerned in transmission. The parasites were identified to subgeneric level, and at least six to species. Double and triple infections were common in high density, so that a large number of gametocytes of several species might circulate at the same time in the blood and be taken up by the mosquito. In this situation, hybridisation was considered to be possible and could account for the minor morphological differences which often made identification impossible. Such crosses have been confirmed in rodent malaria parasites of closely related species, and in conditions of holoendemicity in nature may account for much speciation, provided that a degree of isolation exists.

Multiple infections may even extend to two species being present in a single host cell. Recently Young *et al.* (1979) described three species of parasites — *Haemoproteus meleagridis*, *Leucocytozoon smithi*, and *Plasmodium hermani* — in sentinel turkey poults suspended in cages high up in the swamp Cypress (*Taxodium distichum*) trees. During the peak of parasitaemia, a *Leucocytozoon* gametocyte and a trophozoite of *Plasmodium* were sometimes present in one host cell.

6. *Multiple infections of parasites in geckoes.* During a visit to Rioux's territory in the South of France in August 1978, I was able to participate in the interesting work of the Equipe franco-britanique on leishmaniasis and particularly on the parasites of *Tarentola mauritanica* around Banyuls. This tiny lizard harbours in its blood 5 different organisms — several if not all may be present in the same specimen. *Leishmania tarentola* was actually seen in the amastigote form in the blood and promastigotes were cultured from the heart blood of 20 % of the geckoes (Rioux *et al.* 1978). *Haemocystitium tarentola* gametocytes were present in small or large numbers in many specimens; we tried unsuccessfully to get the males to exflagellate (even with the aid of the special head factor of

Carter & Nighout) and equally unsuccessfully to infect *Sergentomyia minuta* or *Culicoides nubeculosus* (kindly supplied by Mme. Landau from Paris) by feeding through a membrane on positive geckoes. Trypanosomes were found in two geckoes, one in division. Sporozoites of *Schellackia* were seen in several specimens ; mites were collected from these lizards, fixed and sections examined, but no development was found. Of more interest was the frequent presence of *Pirhemocyton* in many erythrocytes, and as this virus-like organism (as confirmed later by electron microscopy) has been shown to have a deleterious effect on intracellular parasites, it is remarkable that they should co-exist in this locality.

7. *Virus infections.* Apart from *Pirhemocyton*, there are many instances, of other viruses accompanying blood and intestinal protozoa in vertebrates (and in the host). Most of my experience has been with mosquitoes infected experimentally with various species of *Plasmodium* and infected accidentally with the dreaded cytoplasmic polyhedrosis virus. A quick look at the midgut reveals the presence of yellowish or brown amorphous masses. This virus has a profound effect on sporogony of the *Plasmodium* (Bird *et al.* 1972) and many experiments were ruined.

In the last few years, a " new " disease of man has been described (Bird & Smith — 1980 — and Bird pers. comm.) in which the causative organism is the coccidian, *Cryptosporidium*, but its pathological effect is exerted only when an adenovirus is present and usually in children with immunodeficiency. The *Cryptosporidium* then fulminates on the epithelium of the colon and rectum, and an intractable, usually fatal diarrhoea is the outcome.

Polyparasitism is extremely common in nature and I have chosen my examples chiefly from malaria parasites, viruses and rickettsia. The different parasites may have to compete with each other for food and the less fit suffer from starvation. On the contrary there may be exacerbation of one parasite by the other.

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NOTES AND QUESTIONS

BY

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A meeting on parasitic specificity hat at least two sides : Host and Parasite ; frequently it is 3-sided : Vertebrate Host, Obligatory Invertebrate Vector and Parasite; while sometimes it is 4-sided, as added to the last combination, may be an Animal Reservoir.

Specificity applies to all these elements. We have innumerable examples of their importance. European strain of *Plasmodium falciparum* is specifically adapted to European species of *Anopheles* : it will not develop in African *A. gambiae* ; and *mutatis mutandis*, African strain of *P. falciparum* will not develop in European *A. atroparvus*.

But my own paper does not deal with this question of strain, deme, subspecies and species, but raises another vexed issue : multiple i.e. mixed infections. I think it would be true to state that *all* vertebrates including man have mixed infections including the flora of the intestinal tract. This applies to animals in temperate zones, but the plethora of species is augmented in the tropics.

The question is what influence does one parasite have on the others ; and how does the combination affect the host — vertebrate or invertebrate ?

To these questions is added another : when two closely allied organisms are present, can hybrisation occur ? If it occurs its site is probably the midgut of the vector when fertilisation takes place *vide* Walliker's experiments, Gabaldon's in the field. Or, a more remote possibility, will the presence of one organism increase the chances of mutation in another ?

My field is more or less limited to arthropod-transmitted haematozoa and very much directed to the malaria — or malaria-like parasites and arthropod vectors. May I first briefly mention the tick or mite — transmitted infections, because these differ from the rest in one fundamental character — the invertebrate host is the real host i.e. the reservoir — it is not just a vector of the infection (as is the case with all the rest), but offers a permanent home to the parasite, e.g. spirochetes in argasid ticks, piroplasms in hard ticks, rickettsiae in mites. Even when classical cycles occur in the tick, like *Hepatozoon* in *Rhipicephalus*, the parasite lingers in the tick sometimes long after the dog has shaken it off. Moreover, the tick in some cases is usually specific, while many species of vertebrate animal may be susceptible. One other point before I leave ticks and their parasites : the ubiquity of both, at least in mammals. This suggests ancient lineage.

Although not relating directly to my own paper, the texts of M. Fain and of M. Hofstetter have stimulated me to pose certain questions on problems which have long puzzled me. These may already have been discussed by Dr. Baker and Mme. Landau, but perhaps, my approach is slightly different from theirs.

The questions relate to the baematozoa and are in 2 categories :

1. Distribution of parasites according to Order, Family or Species of vertebrate host.

2. Zoogeography i.e. distribution of parasite, vertebrate hosts, arthropod vector, reservoir host.

1. Distribution of parasites is very uneven — trypanosomes and piroplasms are widespread throughout the vertebrates (mammals, birds, reptiles, amphibia and fish), while the haemogregarines come a good third.

But the Haemosporidia show remarkable preferences. Let us look at the mammalian families in which they are *absent* : in the lemuroids except in the true lemurs ; in all ungulates, except a few special forms (chevrotains, water buffalo, deer, duikers) ; in marine vertebrates ; in all carnivores ; in many rodents, except for certain squirrels, murids and *Anomalurus* and *Atherurus* ; in edentates ; in most insectivores except the elephant-shrews (2 genera) ; in marsupials and other Australasian mammals. Haemosporidia are essentially parasites of primates, and less of certain genera of bats, rodents and other arboreal small mammals.

The insect hosts of the Haemosporidia are Diptera — notably mosquitoes and ceratopogonids, in which groups of certain species are particularly concerned e.g. the *leucosphyrus* group of *Anopheles* in simian malaria parasites.

I will not discuss the avian and saurian Haemosporidia except to point out that nearly all families of birds are extensively parasitised. The International Avian Malaria Centre (Gordon Bennett) in Newfoundland is deeply occupied in the systematics of this subject. The reptile parasites are interesting in that the distribution in the various groups is strikingly different — as follows :

Lizards +++ in all families of Haemosporidia.
Chelonian (fresh water turtles only) ++ in one group only (*Simondia*).
Snakes. Very rare.
Crocodilia. Absent.

The subject of saurian malaria is being extensively studied by Telford in North, Central and South America, in Pakistan, S.E. Asia and Japan and Africa. He has recently shown (1981) that a single species (*Plasmodium sasai*) has the widest distribution in three species of *Takydromus* from Japan to Thailand, where the malaria is associated with at least 3 other parasites. He suggests that the presence of this complex in a single genus of lacertiliids represents concomitant evolution from ancestral stock.

2. My second category relates to problems of zoogeography. The geographical distribution is equally uneven — trypanosomes, piroplasms and haemogregarines are again widespread in all continents, the Haemosporidia show remarkable features ; and again I will concentrate on the forms present in mammals, as the parasites of birds and reptiles are very different. For instance, *Plasmodium* (*Haemamoeba*) *relictum*, *Plasmodium* (*Carinamoeba*) *minasense*, *Haemoproteus columbae*, *Simondia metchnikovi* have a cosmopolitan distribution. The lists indicate the place of origin and hosts of the mammalian species.

There are 5 points in the lists to which I wish to draw particular attention, and ask for suggestions :

1. Excluding peripatetic Man and his malaria parasites there is an almost complete absence of all 3 subgenera of *Plasmodium* in the New World mammals. *P. brasilianum* is common in the New World monkeys, but this species seems to be identical with the human *P. malariae* which was probably imported and infected mosquitoes and monkeys in turn. The rare *P. simium* may represent a similar introduction of the human *P. vivax*.

2. Why are the haemoproteid parasites so rare in America ? *Hepatocystis* is entirely absent and the common parasite of bats is only known from the Amazon and California (in both places in the Old World genus *Myotis*).

3. Why is *Plasmodium* absent from all the African monkeys except for small foci in mangabeys and mandrilil on the West Coast ? Yet the human species are common in the chimpanzees and gorillas, and the Asian monkeys of all kinds are heavily parasitised.

4. Why are the haemoproteid parasites entirely absent from man and the Great Apes ?

5. Why is *Plasmodium* absent from wild rodents except in West and Central Africa ? And why are the Haemosporidia in general so poorly represented in the other mammals except for a few groups — essentially the primates — less in the squirrels and bats. Why are carnivores and marsupials entirely free ?

I stress this patchy distribution in mammals. It is commonly thought that the Haemosporidia stemmed from the Coccidia of the intestine of the remote ancestor. It is curious, however, that the Coccidia are the reverse of patchy today — occurring in nearly all mammals. Why did so few manage to escape from the gut into the blood ? Perhaps a suitable insect vector was not available.

There are 2 possible factors in these problems : 1, continental drift — measured in tens of millions of years and 2, ice ages (with complete interruption of insect transmission) measured in only tens of thousands of years. Their mechanics and relative importance is conjectural.

DISCUSSION

Sprent. — In view of the possibility of antagonism between *Plasmodium* and *Babesia*, is there any possibility that the absence of *Plasmodium* in certain mammalian groups is related to the presence of *Babesia* ?

Garnham. — We cannot be sure. Experimental results have often indicated a refractility to infection.

Landau. — *Thamnomys* est parasité spontanément par les deux genres.

Léger. — Cela dépend-il de la température ?

Czaplinski. — What is the mechanism of the prophylactic effect of the two *Plasmodium* species, *P. vivax* and *P. falciparum* on *Treponema pallidum* ? Many years ago *Plasmodium* was used for control of syphilis, but was it only because of high temperature ?

Garnham. — The mechanism is still not clear.

Kim. — Looking at the distribution of *Plasmodium* in anthropoids, we may ask how and when *Plasmodium* invaded the arthropods or even the mammals ?

Garnham. — The great Apes of Africa have similar species and might have a common origin.

Lavocat. — On peut distinguer 2 groupes de Rongeurs parasités par les *Plasmodium* :

1) Un groupe qui n'est pas arrivé en Afrique avant la fin du Miocène.
2) Les Anomalures, qui sont probablement en Afrique depuis l'Eocène.

Chabaud. — D'accord avec M. Lavocat : il y a 2 sortes de Rongeurs parasités.

1) Les hôtes « véritables » Athérures et Anomalures qui sont anciens.

2) Les hôtes modernes *Thamnomys* et *Grammomys*, donc pratiquement 1 seul genre parmi les dizaines de Muridés africains. C'est une capture ponctuelle et récente provenant du fait qu'ils sont arboricoles.

Ainsi que l'a déjà écrit Irène Landau, les Vertébrés très « anciens » (Insectivores — Marsupiaux) et les Vertébrés très « modernes » (Muridae, Arvicolidae, Bovidae) sont presque tous indemnes. Le spectre d'hôtes des Plasmodium de Mammifères semble correspondre aux animaux qui ont des ancêtres, semblables aux représentants actuels, apparus aux environs de l'éocène.

En outre, les entomologistes paraissent d'accord pour admettre que le genre *Anopheles* est postérieur aux Culicinae, et a pu également apparaître à cette époque.

Pour les Plasmodium de Mammifères, comme pour les Nématodes, ce serait donc avant tout la période géologique qui détermine le spectre d'hôtes et non les affinités zoologiques réciproques de ceux-ci.

Hoffstetter. — Il est curieux que, de tous les Lemuriformes, seule une espèce de *Lemur* soit attaquée par un Plasmodium.

L'enquête a-t-elle été faite sur les autres Lémuriens de Madagascar, les *Daubentonia*, les Pottos et les Galagos d'Afrique, les Loris asiatiques ? Si cette enquête a eu lieu et qu'elle est négative, y a-t-il une explication ?

Brygoo. — De nombreuses recherches ont été faites sur les Lémuriens malgaches, en particulier sur le Microcèbe et le Cheirogale ; elles sont négatives. Ces animaux sont, en tout cas, peu infestés.